We claim:

1. A composition comprising a substantially purified thermostable Gux1 peptide, said Gux1 peptide comprising a catalytic domain GH48, a carbohydrate binding domain (CBD) type III, and a carbohydrate binding domain (CBD) type II.

2. The composition of claim 1 wherein the Gux1 peptide is further defined as comprising a linker and a signal peptide.

3. The composition of claim 1 or 2 wherein the GH48 catalytic domain of the Gux1 peptide is further defined as having a length of about 637 to about 643 amino acids.

4. The composition of claim 1, 2, or 3 wherein the carbohydrate binding domain (CBD) type III of the Gux1 peptide is further defined as having a length of about 150 to about 156 amino acids.

5. The composition of claim 1, 2, 3, or 4 wherein the carbohydrate binding domain (CBD) type II of the Gux1 peptide is further defined as having a length of about 95 amino acids to about 105 amino acids in length.

6. The composition of claim 3 wherein the GH48 catalytic domain is further defined as the sequence of SEQ ID NO: 5.

7. The composition of claim 4 wherein the carbohydrate binding domain (CBD) type III is further defined as the sequence of SEQ ID NO: 4.

8. The composition of claim 6 wherein the carbohydrate binding domain (CBD) type II is further defined as the sequence of SEQ ID NO:7.

9. The composition of claim 1 further defined as comprising a sequence of SEQ ID NO: 4, SEQ ID NO: 5, and SEQ ID NO: 7.

10. A thermal tolerant Gux1 peptide having a sequence of SEQ ID NO: 1.

11. The Gux1 peptide of claim 10 further defined as having a sequence of SEQ ID NO: 2.

12. An industrial mixture suitable for degrading cellulose, such mixture comprising the Gux1 polypeptide of claim 1.

13. The industrial mixture of claim 12 further defined as comprising a detergent.

14. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.

15. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 80% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.

16. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 70% sequence identity to the nucleic acid sequence encoding an amino acid sequence of SEQ ID NO: 5.

17. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 7.

18. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 4.

19. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 6.

20. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% sequence identity to the nucleic acid sequence encoding the amino acid sequence of SEQ ID NO: 1.

21. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence having at least 90% identity to the nucleic acid sequence of SEQ ID NO: 2.

22. The composition of claim 1 wherein the Gux1 is further defined as comprising a nucleic acid sequence encoding a heterologous protein in frame with the Gux1 peptide of claim 1.

23. The composition of claim 22 wherein the heterologous protein in frame with the Gux1 peptide of claim 1 is further defined as a peptide tag.

24. The composition of claim 23 wherein the peptide tag is 6-His, thioredoxin, hemaglutinin, GST, or OmpA signal sequence tag.

24. The composition of claim 22 wherein the heterologous protein is a substrate targeting moiety.

25. The composition of claim 13 wherein the nucleotide sequence encoding the Gux1 is operably linked to a transcriptional or translational regulatory sequence.

26. The composition of claim 25, wherein the transcriptional or translational regulatory sequence comprises a transcriptional promoter or enhancer.

27. An isolated polypeptide molecule comprising:

a) a sequence of SEQ ID NO: 4;

b) a sequence of SEQ ID NO: 5;

c) a sequence of SEQ ID NO: 6;

d) a sequence of SEQ ID NO: 7;

e) a sequence of SEQ ID NO: 1; or

f) an amino acid sequence having at least 70% sequence identity with the amino acid sequence of a), b), c), d), or e).

28. The polypeptide molecule of claim 27, having at least 90% sequence identity with the amino acid sequence of a), b), c), d), or e).

29. A fusion protein comprising the polypeptide of claim 27 and a heterologous peptide.

30. The fusion protein of claim 29, wherein the heterologous peptide is a substrate targeting moiety.

31. The fusion protein of claim 29, wherein the heterologous peptide is a peptide tag.

32. The fusion protein of claim 31, wherein the peptide tag is 6-His, thioredoxin, hemaglutinin, GST, or OmpA signal sequence tag.

33. The fusion protein of claim 29, wherein the heterologous peptide is an agent that promotes polypeptide oligomerization.

34. The fusion protein of claim 29, wherein the agent is a leucine zipper.

35. A cellulase-substrate complex comprising the isolated polypeptide molecule of claim 27 bound to cellulose.

36. A vector comprising the polynucleotide molecule that encodes a polypeptide of claim 27.

37. A host cell genetically engineered to express the polypeptide molecule of claim 27.

38. A host cell genetically engineered to express the polynucleotide molecule of claim 27.

39. The host cell of claim 37 or 38, wherein the host cell is a plant cell.

40. The host cell of claim 37 or 38, wherein the host cell is a fungi.

41. The host cell of claim 37 or 38, wherein the host cell is a bacterial cell.

42. The host cell of claim 37 or 38, wherein the host cell is a bacterial cell.

43. A composition comprising the polypeptide molecule of claim 27 and a carrier.

44. A composition comprising the polypeptide molecule of claim 28 and a carrier.

45. An isolated antibody that specifically binds to the polypeptide molecule of claim 27.

46. The antibody of claim 45, wherein the antibody is a polyclonal antibody.

47. The antibody of claim 45, wherein the antibody is a monoclonal antibody.

48. A method for producing Gux1 polypeptide, the method comprising:
incubating a host cell genetically engineered to express the polynucleotide molecule of claim 27.

49. The method of claim 48, further comprising the step of:
isolating the Gux1 polypeptide from the incubated host cells.

50. The method of claim 48, wherein the host cell is a plant cell.

51. The method of claim 48, wherein the host cell is a bacterial cell.

52. The method of claim 48, wherein the host cell is genetically engineered to express a selectable marker.

53. The method of claim 48, wherein the host cell further comprises a polynucleotide molecule encoding one or more polypeptide molecules selected from the glycoside hydrolase family of proteins.

54. The method of claim 53, wherein the glycoside hydrolase is a thermostable glycoside hydrolase.

55. A set of amplification primers for amplification of a polynucleotide molecule encoding Gux1, comprising:

two or more sequences comprising 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27.

56. A probe for hybridizing to a polynucleotide encoding Gux1, comprising:

a sequence of 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27.

57. An assay method for the detection of a polynucleotide encoding Gux1, comprising:

amplifying a nucleic acid sequence with a set of amplification primers comprising two or more sequences of 9 or more contiguous nucleic acids derived from the polypeptide molecule of claim 27; and

correlating the amplified nucleic acid sequence with detected polypeptide encoding Gux1.

58. A method for assessing the carbohydrate degradation activity of Gux1 comprising:

analyzing a carbohydrate degradation in the presence of Gux1 and a carbohydrate degradation in the absence of Gux1 on a substrate; and

comparing the carbohydrate degradation in the presence of Gux1 with the carbohydrate degradation in the absence of Gux1.

59. A method for assessing the carbohydrate degradation activity of Gux1 in the presence of an agent of interest comprising:

analyzing a carbohydrate degradation in the presence of Gux1 and a carbohydrate degradation in the presence of Gux1 and the agent of interest on a substrate exposed; and

comparing the carbohydrate degradation in the Gux1 treated substrate with the carbohydrate degradation in the Gux1 treated substrate in the presence of the agent of interest.

60. The method of claim 59, wherein an increase in carbohydrate degradation activity in the presence of the agent of interest demonstrates stimulation of Gux1 activity and wherein a decrease in carbohydrate degradation activity demonstrates inhibition of Gux1 activity.

61. The method of claim 58, wherein the carbohydrate is cellulose.

62. The method of claim 58 wherein the agent of interest is an antibody.

63. A method for reducing cellulose in a starting material, the method comprising:

administering to the starting material an effective amount of a polypeptide molecule of claim 27.

64. The method of claim 63, further comprising administering a second polypeptide molecule selected from the glycoside hydrolase family of proteins.

65. The method of claim 63, wherein the polypeptide molecule of claim 27 is thermostable.

66. The method of claim 63, wherein the starting material is agricultural biomass.

67. The method of claim 63, wherein the starting material is municipal solid waste.

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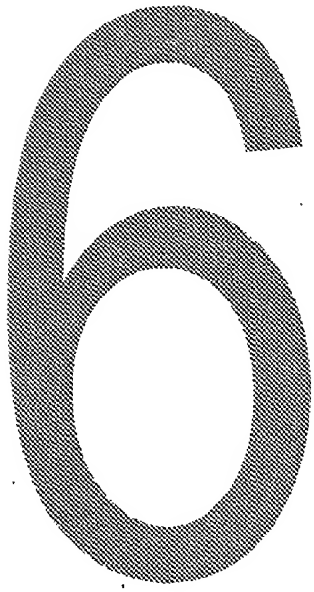
Abstract



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Abstract of the Disclosure

The invention provides a thermal tolerant cellulase that is a member of the glycoside hydrolase family. The invention further discloses this cellulase as Gux1. Gux1 has been isolated and characterized from Acidothermus cellulolyticus. The invention further provides recombinant forms of the identified Gux1. Methods of making and using Gux1 polypeptides, including fusions, variants, and derivatives, are also disclosed.

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Drawings

7

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Fig. 1.

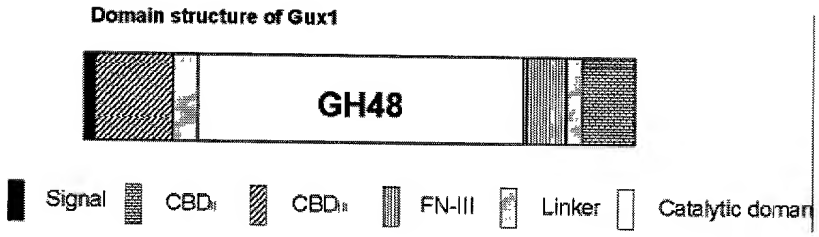
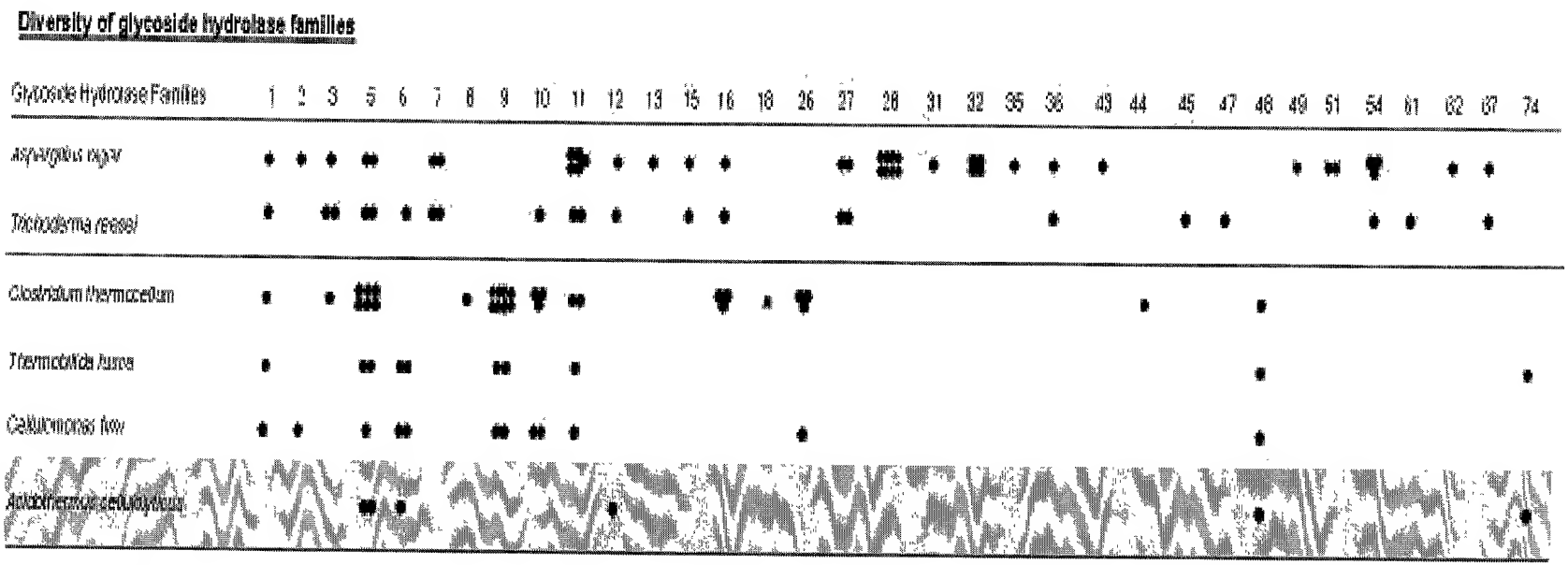

Domain structure of Gux1
GH48
Signal
CBDI
CBDII
FN-III
Linker
Catalytic doman

FIG. 2


Diversity of glycoside hydrolase families
Glycoside Hydrolase Families
1 2 3 5 6 7 8 9 10 11 12 13 15 16 18 26 27 28 31 32 35 36 43 44 45 47 48 49 51 54 61 62 67 74
Aspergillus niger
Trichoderma reesei
Clostridium thermocellum
Thermotoga maritima
Cellulomonas fimi
Actinomyces cellulolyticus

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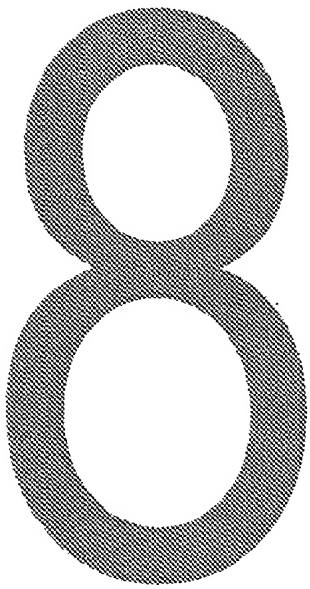
Oath/Declaration, Small Entity, and Power of Attorney



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COMBINED DECLARATION AND POWER OF ATTORNEY

As the below named inventor(s), I (we) hereby declare that:

My (Our) residence, post office address and citizenship(s) are as stated below next to my (our) name(s).

I believe I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled

THERMAL TOLERANT EXOGLUCANASE FROM ACIDOTHERMUS CELLULOLYTICUS

the specification of which (check one)

☒ is attached hereto ☐ as filed on _____ as Serial No. _____

and was amended _____

I (We) hereby state that I (we) have reviewed and understand the contents of the above-identified specification, including claims, as amended by any amendment referred to above.

I (We) acknowledge the duty to disclose information which is material to the examination of this application in accordance with Title 37, Code of Federal Regulations, § 1.56(a).

I (We) hereby claim foreign priority benefits under Title 35, United States Code § 119 of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed:

PRIOR FOREIGN APPLICATION(S)

Number	Country	Filed (Day/Month/Year)	Priority claimed Yes	Priority claimed No
			☐	☐

I (We) hereby claim the benefit under Title 35, United States Code, § 120 of any United States application(s) listed below and insofar as the subject matter of each of the claims of this application is not disclosed in the prior United States application in the manner provided by the first paragraph of Title 35, United States Code, § 112, I (we) acknowledge the duty to disclose material information as defined in Title 37, Code of Federal Regulations, § 1.56(a) which occurred between the filing date of the prior application and the national or PCT international filing date of this application:

Serial No.	Filing Date	Status Pending

POWER OF ATTORNEY: As the named inventor(s), I (we) hereby appoint the following attorney(s) and/or agent(s) to prosecute this application and transact all business in the Patent and Trademark Office connected therewith.

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Paul J. White Senior Counsel National Renewable Energy Laboratory 1617 Cole Boulevard Golden, CO 80401	Paul J. White 303/384-7575

We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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We hereby declare that all statements made herein of our own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

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Residence _____ Citizenship _____

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Full name of joint inventor _____

Inventor's signature _____ Date _____

Residence _____ Citizenship _____

Post Office Address _____

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Full name of joint inventor _____

Inventor's signature _____ Date _____

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Post Office Address _____

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